

Double-dipping does transfer bacteria: George was wrong!



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INTRODUCTION: While casually mingling at some social event you suddenly catch someone performing the infamous “double-dip”—the redipping of a chip that has already been bitten off of. The type of chip and the type of dip are irrelevant to the act that many people consider impolite and unsanitary. Yet, others see no crime in the double-dip and feel no shame in dunking the same chip two, three, or even four times in the dip. Whether the act is rude or not, the real question remains: Is the double-dip concern plausible? How much bacteria is actually transferred from the culprit mouth to the victimized dip? The Centers for Disease Control (<http://www.cdc.gov/flu/proteccovercough.htm>) recommend covering the mouth to prevent spreading “serious respiratory illnesses like influenza, respiratory syncytial virus (RSV), whooping cough, and severe acute respiratory syndrome (SARS).” While coughing, sneezing and touching contaminated surfaces are vectors for spreading disease, the disease agents are carried in the mucus of infected persons (<http://www.health.state.ny.us/publications/7110/>). Bacterial cells in the mouth attach to each other to form dynamic bacterial communities (Kolenbrander, et al 2002). Most of these oral bacteria communities reside on the teeth and eventually develop into plaque. Human oral bacteria are not naturally found anywhere else in the body. Outside of the mouth, oral bacteria are considered pathogenic. Previous studies’ estimates of the number of oral bacterial species range from 500 types (Kolenbrander, et al 2002) to 700 types (Aas, et al 2005). According to Micik et al (1969), coughing, sneezing, or even simply speaking can cause oral bacteria to become airborne. Particles that are small enough in size can act as aerosols and then linger in the air, easily spread to other individuals. Bacterial aerosol counts for speaking and breathing were low; however, for coughing and sneezing, Micik et al (1969) observed median bacterial aerosol counts of 36 CFU/min and 550 CFU/min, respectively. If bacteria can be transferred among individuals from the mouth through the air, then surely it can also be transferred by the contamination of food with multiple individuals’ saliva. The objective of this study was to determine if dipping a cracker after biting the cracker would transfer bacteria from the mouth to a solution.

MATERIALS & METHODS: Prior to conducting the dipping experiment, total bacteria in the mouth was measured by rinsing each student’s mouth with 20 ml of sterile water which was then serially diluted and plated in duplicate on nutrient agar. After incubation for 48 hours at 38 C, plates from dilutions with between 25-250 colony forming units (cfu) were counted and converted to log cfu per ml of the rinse solution. Wheat thins were used as the food product and 20 ml of sterile water acted as the dipping solution. Each student in the CI team conducted four treatments. For the dipping treatments, a cracker was bitten, dipped in the sterile water then discarded (Figure 1). The control treatments consisted of dipping a cracker without biting. The four treatments were: 3 dips without biting, 6 dips without biting, 3 dips with biting, and 6 dips with biting. Each of the 8 students then took a 1 ml sample from each dipping solution which was serially diluted then plated in duplicate (Figure 2). Again, after incubation for 48 hours at 38 C, plates from dilutions with between 25-250 colony forming units (cfu) were counted and converted to log cfu per ml of the dipping solution.



Figure 1. Dipping wheat thins in sterile water.

Figure 2. Dilution and plating of samples.



Statistical Analysis: The “double-dipping” experiment was replicated 8 times and all plating was in duplicate. The dipping treatments were subjected to an analysis of variance (ANOVA) using SAS (2006) to determine if there was a significant ($P \leq 0.05$) overall effect due to these treatments. Since there was a significant effect, the means were separated using the pdiff command of SAS.

RESULTS: Oral cavity bacterial counts for the team members averaged 6 log cfu/ml of rinse with little variation among the group (Table 1). According to Kang et al. in a study titled *Bacterial diversity in the human saliva from different ages*, fourteen genera were identified in human saliva. *Streptococcus*, *Prevotella*, and *Veillonella* were among the genus most common to the human oral cavity with *Streptococcus* being the predominant genus. Future research plans to identify the genus of bacteria being transferred to the dipping solution. For the “double dipping” experiment, a higher population of bacteria ($P \leq 0.05$) was found in solutions dipped with crackers after biting compared to solutions dipped without biting (Figure 3). There was no difference between the 3 and 6 dips ($P > 0.05$) as far as bacteria transferred to the dipping solution. Bacterial populations found in the solution after crackers were dipped without biting were less than 10 cfu per ml of the dipping solution. The results of our research proved that bacteria can be transferred from the mouth to the dip.

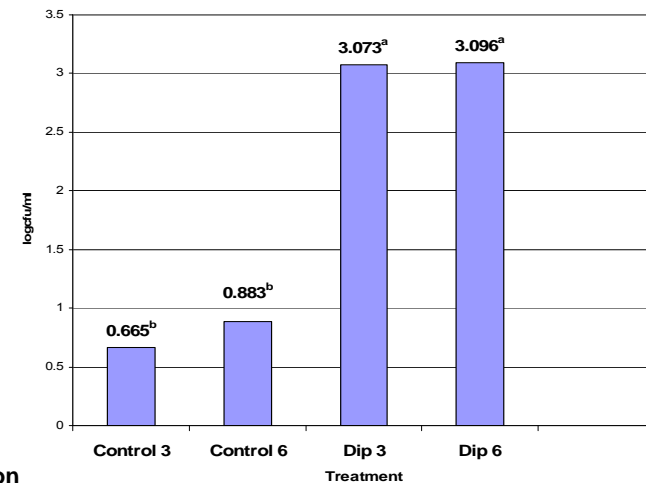
Table 1. Total oral bacterial population for 7 subjects in the study

Subject	log cfu/ml
1	6.10
2	6.34
3	6.35
4	6.69
5	5.98
6	5.76
7	5.83
Mean (SD)	6.26 (.31)

DISCUSSION: To analyze the true effect of double-dipping, Clemson University’s Dr. Paul Dawson and his Creative Inquiry Team (Testing Variables of Foods, Films, Antimicrobials and Surfaces Affecting Transfer and/or Survival of Bacteria) conducted an experiment with the purpose of determining the extent of bacteria transfer that occurs when someone double-dips. The inspiration of the experiment was a popular television episode of Seinfeld, written by Peter Mehlman, in which the character George Costanza is scolded for double-dipping. First, mouth rinses were performed to measure the average bacteria count present in the mouth under normal conditions. Oral bacterial counts ranged from 5.8×10^5 — 4.8×10^6 CFU/mL (5.8 — 6.7 logCFU/mL) and averaged 1.8×10^6 CFU/mL. Second, a series of trials were completed that involved biting a chip and then dipping it in sterile water to determine how much bacteria was transferred from the mouth to the chip and then to the dip. Trials included varying amounts of double-dips and a range of dilutions. In order to observe simply the amount of bacteria transfer, sterile water was chosen as the “dip” and low sodium crackers as the “chips” so that the amount of bacteria in the plates would not be influenced by any factors other than the actual transfer from mouth to chip to dip.

According to the data, a significant amount of bacteria transfer occurred that was attributable to double dipping. Bacterial counts averaged 2.6×10^3 CFU/mL (3.4 log CFU/mL) for six dips and 1.9×10^3 CFU/mL (3.3 logCFU/mL) for three dips. It appears that double-dipping does, in fact, contaminate the dip. Whether the amount of contamination is dangerous to the dippers’ health or not is still debatable and can depend on multiple external factors including the type of chip/dip or the relative health/illness of the person whose mouth provided the bacteria. Next time you take a bite of your chip, however, and are tempted to commit a second dip, keep in mind that the numbers have been calculated, and the bacteria are having just as much of a party as you are.

Figure 3. Log cfu/ml of bacteria recovered from sterile water in which crackers had been dipped 3 or 6 times with or without being bitten before dipping. Means shown with the same superscript are not significantly different ($P > 0.05$). The standard error of the means was 0.1.



Mentor: Paul L Dawson